Dextromethorphan Analogs: Receptor Binding and Pharmacological Profile of Novel Anticonvulsant/Neuroprotective Drugs

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ABSTRACT

A series of 3-substituted 17-methylmorphinan analogs of dextromethorphan (DM) have been developed which are anticonvulsant against maximal electroshock seizures in rats. These findings have been extended to a rat model of NMDA convulsions where it has been determined that the aniline (AHN649), O-ethylether (AHN1036) and O-isopropylether (AHN1037) analogs are anticonvulsant. Recent assessments of neurologic impairment have shown that these DM analogs also display exceptional side-effect profiles, yielding protective indices several fold greater than those established for other standard anticonvulsant drugs. In *in vitro* and *in vivo* models of neuronal injury we have determined that AHN649 is at least equipotent to DM as a neuroprotectant. Finally, analysis of binding potencies has revealed no appreciable activity at the non-competitive PCP/MK801 site or the glycine modulatory site of the NMDA receptor complex. Alternatively, interactions with distinct high affinity DM binding sites in brain appear to be involved. We propose that these potent, safe analogs of DM may be of potential therapeutic utility as adjuncts in the treatment of agent-induced convulsions and neurotoxicity.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council.

The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5)

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INTRODUCTION

The antitussives dextromethorphan (DM), caramiphen and carbetapentane have distinguished themselves as anticonvulsant drugs (1) and protective agents against soman-induced convulsions (2). In a search for more potent and efficacious anticonvulsant agents, 3-substituted 17-methylmorphinan analogs of DM have been synthesized and successfully identified as efficacious and potent anticonvulsants in the rat maximal electroshock (MES) model of generalized convulsions (3).

The present report describes our continued efforts on the development of 3 of these potential anticonvulsant compounds; namely the aniline (AHN649), the O-ethylether (AHN1-036) and the O-isopropylether (AHN1-037) derivatives of DM (3). Their pharmacological assessment as anticonvulsants has been extended to two additional seizure models as well as a rotorod model of neurological impairment. In addition, using rat primary neuronal cultures and a rat model of severe forebrain injury, *in vitro* and *in vivo* studies have been initiated to determine the potential neuroprotective properties of these DM analogs. Finally, *in vitro* binding studies are underway in an effort to characterize their receptor binding profile.

METHODS

SPREADING "MES" CONVULSIONS:

Generalized tonic-clonic convulsions were induced in S.D. rats (175-225 g) using transauricular current stimulation (150mA, 2 s). The incidence of tonic hindlimb extension was quantified.

THRESHOLD "FLUROTHYL" CONVULSIONS:

The onset to clonic seizures with loss of posture, i.e. the seizure threshold, was determined using the volatile convulsant flurothyl. Flurothyl was infused as a 10% solution in 95% ethanol (v/v) to S.D. rats (250-300 g) placed individually in sealed, airtight observation chambers.

NMDA CONVULSIONS:

Clonic "popcorn" convulsions were induced in S.D. rats (200-300 g) using a 5 µl i.c.v. injection of 12.5 Nm of the excitatory amino acid (EAA) NMDA. Incidence and latency to convulsion were recorded.

ROTOROD PERFORMANCE:

Neurological impairment was assessed in S.D. rats (260-370 g) trained to remain for 60 s on a rotorod rotating at 6 rev/s.

IN VITRO NEURONAL INJURY:

Primary cultures of "cortical" neurons were established from embryonic day 15 (e15) rats. Glutamate-induced neuronal injury (50 μM glutamate for 45 min) was quantified in 12-15 day old neurons using LDH assessments of cell death.

IN VIVO NEURONAL INJURY:

Severe forebrain injury was induced in S.D. rats (300-400 g) using 15 min of 4-vessel occlusion (Pulsinelli method) followed by 72 h reperfusion. Brains were perfusion fixed (10% formalin) at 72 h post-occlusion, paraffin embedded and processed for quantitative histological assessment (Nissl Stain) of hippocampal CA1 damage using a Loats Image Analysis System.

IN VITRO RECEPTOR BINDING STUDIES:

See legend to Table 6 and reference 3 for methodological details of the various receptor binding assays.

RESULTS

All the DM analogs were more potent than DM against MES convulsions (table 1) while two, AHN1-036 and AHN1-037, were equipotent to the anticonvulsant drugs diazepam and dextrorphan (DX). The analogs were inactive in the rotorod performance test resulting in protective indices (PIs) greater than 14-26, greatly exceeding those calculated for DM, DX or diazepam. Finally, analysis of the anticonvulsant dose-response curves (not shown) demonstrated that the analogs were more *efficacious* than DM in their ability to block MES convulsions.

TABLE 1. MES anticonvulsant and rotorod ED_{50}s (95% confidence intervals) and PIs.

Treatment	MES[a]	Rotorod[a]	PI
Diazepam	1.7 (0.6-5)	6.4 (3-15)	3.8
DM	37.8 (20-72)	229 (55-950)	6.0
DX	4.3 (3-7)	20.6 (14-31)	4.8
AHN649	21.4 (15-30)	>300	>14.0
AHN1-036	5.6 (3-11)	>100	>17.9
AHN1-037	3.9 (2-7)	>100	>25.6

[a]values are expressed as mg/kg.

The DM analogs failed to influence the threshold to flurothyl-induced convulsions (table 2). In contrast, DX was anticonvulsant while DM was *proconvulsant*.

TABLE 2. The effect of the DM analogs on flurothyl seizure thresholds.

Treatment[a]	Dose (mg/kg)	Seizure Threshold (% of control)
Control	--	100%
DM	75	83%*
DX	10	115%*
AHN649	50	101%
AHN1-036	12	98%
AHN1-037	10	98%

[a]All doses represent approximately 2x the respective MES anticonvulsant ED_{50} for each compound.
*P<0.05, Mann-Whitney U Test.

In the NMDA test, DM and the analogs caused a significant, dose-dependent delay in the onset to convulsive behavior. While DM and AHN1-037 failed to attenuate the incidence of NMDA-induced *popcorn* convulsions, AHN649 and AHN1-036 were highly effective (table 3).

Table 3. The effect of the DM analogs on NMDA convulsions.

Treatment[a]	Dose (µg, i.c.v.)	Latency (sec ± s.e.)	% Responding[b]
Control[c]	---	16 ± 0.3	100
DM	20	38 ± 1	83
	40	61 ± 3	83
	80	78 ± 11	100
AHN649	20	58 ± 6	83
	40	61 ± 10	83
	80	98 ± 15	33
AHN1-036	20	43 ± 5	67
	40	66 ± 19	67
	80	120 ± 0	0
AHN1-037	20	30 ± 2	100
	40	48 ± 6	83
	80	81 ± 11	100

[a]all rats received 12.5 nM NMDA (i.c.v.) 15 minutes post-treatment.
[b]% per group (n=6) responding with a popcorn convulsion.
[c]I.c.v. vehicle + NMDA.

In vitro glutamate neurotoxicity was antagonized by DM and AHN649 in a concentration-dependent manner (data not shown); their estimated EC_{50}s were 5 μM and 10 μM, respectively. Preliminary results indicate that the analogs AHN1-036 and AHN1-037 are also neuroprotective, producing 59% and 30% protection, respectively at 25 μM.

Severe forebrain injury resulted in extensive and consistent loss of pyramidal CA1 neurons which could be prevented by post-treatment (1, 2 and 4 h) with 20 mg/kg (s.c.) of DM or AHN649 (table 4).

Table 4. Effect of DM and AHN649 to attenuate CA1 damage associated with severe forebrain injury[a].

Treatment	n/group	% Damage ± s.e.
Sham Occl[b]	4	8 ± 4
Vehicle + Occl	5	75 ± 9
DM + Occl	7	44 ± 14
AHN649 + Occl	7	21 ± 11

[a]All rats had their vertebral arteries occluded and snares placed around the carotid arteries 24 h prior to testing.
[b]Complete surgical procedure but without occlusion of the carotid arteries.

The results of the radioligand displacement studies, while incomplete, suggest that an interaction of DM and the analogs at specific [^{3}H]DM sites may be involved. No appreciable binding of the DM analogs has been measured at sites labelled by [^{3}H]glycine while only AHN649 displaced [^{3}H]TCP with low potency. These studies are summarized in table 5.

DISCUSSION

The purpose of this ongoing study has been to explore the anticonvulsant pharmacology and potential neuroprotective properties of a series of novel analogs of DM. Using rodent models of convulsive behavior, neurological deficit, *in vitro* and *in vivo* neuronal injury, and receptor binding it has been possible to significantly expand our knowledge of the actions of these analogs.

Not only are the analogs potent and efficacious anticonvulsants, we have demonstrated that their PIs greatly exceed those for DM, DX and diazepam, as well as other standard anticonvulsant drugs (4).

Table 5. Binding data for the DM analogs[a].

Compound	IC_{50}, μM[b]		
	[^{3}H]DM (rat brain)	[^{3}H]TCP (rat SPM[c])	[^{3}H]GLY (rat SPM)
DM	0.59 ± 0.12	2.0 ± 0.6	NA[d]
AHN649	45%	7.8 ± 1.4	NA
AHN1-036	0.42 ± 0.06	75%	NA
AHN1-037	0.88 ± 0.18	59%	NA
MK801	42%	0.01 ± .003	10%
PCP[e]	NT	0.18 ± 0.04	NT
GLY[e]	NT	NT	0.69 ± 0.2

[a]Modified from reference 3.
[b]IC_{50} values given are expressed in micromolar where a displacement curve was obtained, or in percent inhibition at 10^{-3} M where this was less than 50%, or where an abnormally high slope was obtained.
[c]Synaptic plasma membrane.
[d]No effect (NA); not tested (NT).
[e]PCP (phencyclidine); GLY (glycine).

In addition, these analogs can be further distinguished from DM since, unlike DM, high doses of the analogs were not proconvulsant, an attribute which probably contributes to the improved anticonvulsant efficacy observed with these compounds. This is a critical point because other anticonvulsant drugs, such as phenytoin and carbamazepine (5), are also proconvulsant at high doses, a potentially limiting factor in treating severe, drug refractory epileptic conditions such as agent-induced seizures.

In view of the possible role of EAA systems as secondary modulators of agent-induced epileptogenesis and subsequent neuronal damage (6,7), we have directly assessed the ability of these analogs to influence convulsant activity and neuronal responses to NMDA (*in vivo*) and glutamate (*in vitro*). The analogs were at least as effective as DM against NMDA-induced convulsions and, in the case of AHN649 and AHN1-036, clearly more effective. Results of the *in*

vitro neuronal culture studies show that, similar to DM, the analogs are neuroprotective against glutamate toxicity. While incomplete, results of the *in vivo* neuronal injury study demonstrate that, at least for AHN649, "post-treatment" with these analogs affords protection against severe brain injury.

Collectively, the results of these studies suggest a definitive role for EAAs in the mechanism(s) of action of the DM analogs. However, it is unlikely that direct interactions with the NMDA receptor complex are involved since the analogs fail to compete with any appreciable affinity for the NMDA receptor complex, including the non-competitive PCP/MK801 site or the glycine modulatory site. Alternatively, it appears more likely that interactions with specific DM sites may be involved in their anticonvulsant/neuroprotective mechanism of action, possibly via an NMDA-receptor modulation of intracellular calcium dynamics. Studies are currently underway to explore this possibility.

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